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Article

HPLC-DAD analysis, antifungal and antioxidant activity of *Solanum dolichosepalum* bitter extracts and fractions

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In memory of José Constantino Pacheco, recently deceased, for being an excellent person, an excellent teacher, and a dedicated researcher, and for always bringing out the best in people.

Solanum dolichosepalum is a plant with anti-infective effects. It is a healing agent and has ethnopharmacological uses. In this study, the antifungal activity of extracts and fractions of this species on *C. albicans* and *F. oxysporum* was evaluated. The antioxidant activity was measured using the ABTS and DPPH methods, and by determining the total content of phenolic compounds. An HPLC-DAD qualitative analysis was carried out to identify phenolic compounds and alkaloids. Pearson's correlation coefficients were calculated. Inhibitory effects were found in all the extracts and fractions on the analyzed microorganisms. *F. oxysporum* was the microorganism most sensitive to the action of *S. dolichosepalum* extracts. All extracts and fractions showed antioxidant activity, with the acetone extract and the acetone fraction being those that generated the best results. The content of total phenolic compounds showed that acetone has a greater affinity with the phenolic compounds present in *S. dolichosepalum*. In this plant, *p*-Hydroxybenzoic, vanillic, ferulic, *trans*-cinnamic, caffeic, *p*-coumaric, and rosmarinic acids were found, as well as theobromine, quercetin, and luteolin. The content of total phenolic compounds was determined to be directly proportional to the inhibition of the ABTS and DPPH radicals, and the inhibition of the analyzed microorganisms. It was determined that the extracts and fractions obtained from *S. dolichosepalum* show antioxidant and antifungal activity.

Keywords: Antifungal activity. antioxidant activity. HPLC-DAD analysis. *Solanum dolichosepalum*. *Candida albicans*. *Fusarium oxysporum*.

INTRODUCTION

Solanum dolichosepalum is a wild plant with ethnopharmacological uses which is found in departments such as Caldas and Boyacá. This plant belongs to the Solanaceae family. Popularly called Frutillo, it is a spontaneous, low montane and montane, rainforest plant, found in the Central Cordillera of Colombia. Both the leaves and fruit are used as a healing agent to remove lice and for the treatment of kidney diseases (Martin *et al.*, 2016). In an ethnopharmacological work, the traditional and empirical use of the species as an anti-infective was reported (Ramírez Cárdenas, Isaza Mejía, Pérez Cárdenas, 2013). Arango *et al.* (2004) found that the ethanolic extracts of this species were active against *C. albicans*. In contrast, Marin *et al.* (2006) reported that aqueous extracts were inactive against the same microorganism, but were active against *Trichophyton*

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rubrum. Martin *et al*. (2016) found antifungal activity of the acetone and chloroform extracts of *S. dolichosepalum* against two strains of *Fusarium oxysporum,* isolated from passiflora specimens. Ramírez Cárdenas *et al.* (2017) reported the antibacterial activity of fractions of *S. dolichosepalum* extracted with 2: 1 ether-ethyl acetate, and with chloroform, against *S. aureus* and *E. coli,* as well as the presence of alkaloids, steroids and/or free triterpenoids, tannins, saponins, flavonoids, and cardiotonic glucosides.

Fusarium oxysporum is a cosmopolitan fungus that exists in many pathogenic forms, parasitizing more than 100 species of gymnosperms and angiosperms, thanks to various mechanisms that the fungus has to overcome the defenses of many plants (De Granada *et al*., 2001). *F. oxysporum* attacks several crops of economic interest in the Cundiboyacense highlands region, such as the lulo (*Solanum quitoense),* tomato *(Solanum lycopersicum),* and snap bean *(Phaseolus vulgaris)* (De Granada *et al*., 2001), producing economic losses of great magnitude for the growers of the region, and thus, decreasing their quality of life. Tomato and snap bean crops are vital for the economy of regions such as Valle de Tenza, Samacá, and their surroundings, and it is necessary to contribute to finding alternatives for the control of this fungus that negatively affects the income of the growers. Additionally, this fungus can also cause some conditions in humans, either by direct contact or by the production of mycotoxins, which in immunosuppressed patients can result in pathologies known as fusariosis (De Granada *et al*., 2001).

On the other hand, *Candida albicans* is a fungus, which in its saprophytic form usually appears as a yeast that can affect the skin, internal organs, and more often, the mucosa (Ramírez Cárdenas *et al*., 2017), and it causes major health problems, both in humans (Segundo, 2006) and domestic animals, such as cats and dogs (Marin *et al*., 2006). Although humans are a natural reservoir of *C. albicans* (Segundo, 2006), problems with impaired immune function can cause candidiasis, a pathology that can consist of mild infections of the mucosa and skin or can also trigger a serious systemic spread, affecting vital organs (Perez *et al*., 2004). Infections caused by yeasts of the genus *Candida* have increased dramatically in recent decades, as a consequence of the progress of modern medicine. This has allowed the survival of critically ill patients, as well as advanced surgeries and treatments with immunosuppressive and cytotoxic compounds, along with broad-spectrum antibiotics, generating resistance to the methods used to control said pathogen (De Bedout *et al*., 2003). Furthermore, the treatment of pathological conditions is frequently affected by factors such as the availability, access, cost, duration, and side effects of medications (Anaya-López *et al*., 2006).

In the literature, we can find studies where the antioxidant activity of extracts is related to the presence of phenolic compounds, thanks to the ability of said compounds to trap (Quideau *et al*., 2011), or inhibit the production of, or stabilize free radicals (Quideau *et al*., 2011; Zadra *et al*., 2012). Furthermore, several studies suggest that the presence and amount of phenolic compounds in extracts may be related to the expression of antifungal (Alves *et al*., 2014; Lee *et al*., 2008) and antimicrobial activity (Daglia, 2012).

The objective of this work was to study the antifungal and antioxidant activity of different types of *S. dolichosepalum* extracts and fractions against *C. albicans* and *F. oxysporum*, as well as to find the correlations between each of the analyzed variables and identify some of the compounds present in extracts and fractions.

MATERIAL AND METHODS

Obtaining extracts

Plant material (leaf) was collected in the vicinity of the Vereda Funza in the municipality of Tinjacá, Boyacá, at an altitude of 2,192 meters above sea level, with the following coordinates: 5 ° 34’07.2” North 73 ° 38’28.6” West. The plant material was dried at room temperature (18 ± 2 ° C, Relative Humidity 65%) for 3 weeks. It was dried and ground, and placed in extraction thimbles, which was performed with Soxhlet equipment using different solvents: dichloromethane (Sigma Aldrich, 99.9%), acetone (Panreac, 99.8%), chloroform (Merck, 99.8%), ethanol (JT Baker, 99.5%), and methanol (JT Baker, 99.8%). The amount of plant material used was 50 ± 2 g, with 500 mL of solvent. The extraction time was

8-12 hours in a room with artificial light. Subsequently, the extracts were concentrated under reduced pressure in a rotary evaporator IKA model RV 10. The dried extracts were stored in amber bottles at -20 ± 2 ° C for further analysis.

Obtaining fractions

In order to obtain simpler matrices, column chromatography was performed on the most effective (acetone) extract, following a method reported by (Sticher, 2008), with some variations. The solvent-free extract was taken, macerated with dry silica (Biopetrolabs), and eluted with hexane, dichloromethane, and acetone, successively. The fractions collected were concentrated in rotary evaporation, to then evaluate the antifungal activity against *F. oxysporum* and *C. albicans,* and the antioxidant activity, following the procedures described above.

Antifungal activity

The *F. oxysporum* strain was provided by the Bioplasma Laboratory, isolated from an *S. quitoense* plant, and its identity was corroborated in the Universidad Pedagógica y Tecnológica de Colombia (UPTC) Biological Control Laboratory. Meanwhile, *C. albicans* was provided by the strain collection of the Universidad de Boyacá (ATCC 10231). The aqueous suspension of *F. oxysporum* (spores) and *C. albicans* (colony) were diluted to achieve the turbidity of 0.5 McFarland standard.

For the determination of the antifungal activity of the extracts, the disc diffusion method on PDA agar reported by Martin *et al.* (2016) was carried out, with some modifications. Fluconazole (Genfar, 0.06 mg/mL) and extraction solvents were used as positive and negative controls; the extracts were all evaluated at a concentration of 1122 mg/mL. Petri dishes were incubated at 27 ± 1 and 36 ± 1 ° C for *F. oxyspotum* and *C. albicans* respectively; subsequently, inhibition zones were measured and the percentage of inhibition was calculated. The minimum inhibitory concentration was the last dilution of each extract that did not allow the growth of microorganisms.

Antioxidant activity

Total phenolic compounds

Total phenolic compounds content (TPC) was measured by the Folin-Ciocalteu method. Initially, a calibration curve of gallic acid was prepared in concentrations of 10, 20, 40, 60, 80, and 100 ppm. The determination was made following the procedure reported by Nossa González *et al.* (2016) with some modifications: 125 µL of the gallic acid standard solution (Panreac, 99% monohydrate) were measured, 0.5 mL of distilled H_2O and 125 µL of the Folin-Ciocalteu reagent (Sigma Aldrich, 2M) were added. The mixture was allowed to react for 6 min and 1.25 mL of Na_2CO_3 at 7% (Merck, 99.9% analytical grade) was added, finally, 1 mL of distilled H_2O was added and it was left to rest for 90 minutes; the readings were performed at a wavelength of 760 nm, in a Genesys 10UV UV (Thermo-Electron) spectrophotometer. Then, the total phenolic compounds content of the extracts (3 mg / mL) was determined in the same way as with the gallic acid standards. Total phenolic compounds content in each extract was expressed in mg equivalents of gallic acid per gram of dry extract (mg GAE/g DE). All determinations were performed in triplicate.

DPPH stable radical test

The antioxidant activity of each extract was determined according to the methodology described by Puertas-Mejía *et al.* (2009), with small modifications. Briefly, an aliquot (20 µ.L) of diluted extract in the extraction solvent (3 mg / mL) was added to 1 mL of a methanol solution of DPPH (Sigma Aldrich) $1x10^{-4}$M. The reaction remained in darkness for 30 minutes, and immediately afterward, the absorbance at 514 nm was measured and the percentage inhibition of the radical was determined. As a standard antioxidant Trolox was used in concentrations of 0.002 to 0.4 mM. The inhibitory concentration at 50% of the radical, IC_{50} (mg/mL) was obtained graphically by successive dilution of each extract. All tests were performed in triplicate.

$ABTS^{+\cdot}$ Cation radical assay

To measure the antioxidant activity by the ABTS method, the procedure reported by Puertas-Mejía *et al*. (2009) was used, with some modifications. The $ABTS^{\bullet+}$ radical cation was generated by the reaction of 38.2 mg of ABTS (Sigma Aldrich, 98% HPLC grade) with 7.2 mg of potassium persulfate (JT Baker, grade analysis) dissolved in 10 mL of deionized water for 24 hours, while in darkness. The $ABTS^{\bullet+}$ radical cation formed was diluted with distilled water to obtain the working solution with an absorbance of 0.760 (± 0.020), at a wavelength of 734 nm. Subsequently, 20 µL of the extract (3 mg/mL) was added to 1 mL of the $ABTS^{\bullet+}$ solution and the absorbance was measured at 7 minutes of reaction, determining the percentage of inhibition of the radical. Trolox was used as a standard antioxidant in concentrations of 0.002 to 0.3 mM and the IC_{50} of the radical (mg/mL) was obtained graphically by successive dilution of each extract. All tests were performed in triplicate.

HPLC-DAD qualitative analysis

The extracts were analyzed by HPLC-DAD to identify some phenolic and alkaloid compounds. The sample preparation consisted of concentrating the extracts and fractions to dryness, with a subsequent liquid-solid extraction, using an acetic acid aqueous solution (0.3%): methanol (50:50), accompanied by sonication (15 minutes), centrifugation, and stirring vortexed (10 minutes). Finally, the extract was filtered and the analysis was performed by liquid chromatography with a diode array detector (HPLC-DAD). As reference materials, the xanthines: caffeine, theobromine, and theophylline; catechins (±) -catechin (C), (-) - epigallocatechin gallate (EGCG), (-) - epicatechin (EC), (-) - epigallocatechin (EGC); the flavonoids: quercetin, naringenin, luteolin, kaempferol, ursolic acid, pinocembrin, carnosic acid, and apigenin; and the phenolic acids: gallic, caffeic, *p*-coumaric, rosmarinic, *p*-hydroxybenzoic, *trans*-cinnamic, ferulic, and vanillic were used. All standards used were purchased from Sigma-Aldrich. The analysis was performed on an Agilent Technologies 1200 Series Liquid Chromatograph (LC) (Palo Alto, California, USA) with a Diode Array Detector (DAD) at $\lambda = 245$ nm. The column used was KITINEX (C18) (Phenomenex), 100 mm x 4.6 mm (d.i.) x 2.6 µm (particle size). The injection was carried out automatically, with an elution gradient as mentioned below: Mobile phase A (Acetic Acid 0.3%), Mobile phase B (Acetonitrile HPLC); from 0 to 13 minutes 95.5% of A, from 14 to 17 minutes 85% of A, from 20 to 28 minutes 78% of A, from 30 to 33 minutes 100% of B, from 34 to 40 minutes 95.5 % of A. A flow of 1 mL/min and a temperature of 35 ° C were used. For the identification of the compounds, retention times, and UV-Vis spectra of the standards in 50 ppm concentration were compared.

Statistical analysis

The statistical program SPSS version 24 of IBM was used to perform an analysis of variance (ANOVA) and Duncan's test (when necessary). The tests of comparison of means and the tests carried out were evaluated with a level of significance of 5% ($p = 0.05$), assuming a completely randomized design. Pearson Correlation Coefficients (PCC) were calculated in the same software.

RESULTS AND DISCUSSION

Antifungal activity: Crude extracts

All extracts of *S. dolichosepalum* showed an antifungal effect against the two microorganisms analyzed, finding a statistical difference between them ($p=0.0001$). The zones of inhibition for each of the extracts studied are shown in Table I. The acetonic and methanolic extracts presented better results on the two microorganisms analyzed, with the exception of the ethanolic extract for *F. oxysporum*. It was also observed that *F. oxysporum* was more sensitive to the antifungal effect of the *S. dolichosepalum* extracts, with the acetone extract being even more effective than the fluconazole standard, with 101.9% inhibition.

TABLE I - Antifungal activity of *S. dolichosepalum* extracts, IZ and %I measured at 1144 mg/mL. Mean ± standard deviation (n = 4). Duncan test, different letters in the same column differ statistically at 5%

	F. oxysporum		*C. albicans*	
	IZ (mm)	**I %**	**IZ (mm)**	**% I**
AE	13 ± 2 [b]	101.9 ± 17.1 [b]	6 ± 2 [b]	43.4 ± 10.4 [b]
ME	7 ± 1 [a]	51.9 ± 7.4 [a]	5 ± 1 [a,b]	32.9 ± 6.6 [a,b]
EE	11 ± 2 [b]	80.8 ± 23.9 [b]	4 ± 1 [a]	29.5 ± 6.6 [a]
CE	4 ± 2 [a]	28.8 ± 11.15 [a]	4 ± 1 [a]	29.4 ± 8.7 [a]
DE	4 ± 2 [a]	30.8 ± 7.4 [a]	3 ± 1 [a]	20.8 ± 5.7 [a]
FLU	13 ± 1		14 ± 2	

AE: Acetone extract; ME: methanolic extract; EE: ethanolic extract; CE: chloroform extract; DE: dichloromethane extract; FLU: fluconazole; I %: inhibition percentage; IZ: inhibition zones.

Many investigations have mentioned the effectiveness of extracts isolated from various species of the genus Solanum against these microorganisms, however, we will focus on reports of just one species. Preliminary results with acetonic and chloroform extracts of *S. dolichosepalum* leaf were published in 2016; two strains of *F. oxysporum* isolated from two specimens of passion fruit (*Passiflora ligularis*) were used. It was found that the acetone extract was more active against strain 1 (20 ± 2 mm zone of inhibition and 95 ± 9% inhibition), while the chloroform extract was more effective against strain 2 (18 ± 1 mm zone of inhibition and 94 ± 6% inhibition) (Martin *et al*., 2016). These cited results are greater than those found, given that the *F. oxysporum* strain used has a different origin, which is why sensitivity is possibly affected by environmental and nutritional inductions generated by the host (De Granada *et al*., 2001). Ramírez *et al.* (2017) report the antibacterial activity of fractions of *S. dolichosepalum* extracted with 2:1 ether-ethyl acetate and with chloroform against *S. aureus* and *E. coli*. Ortíz *et al.* (2019) reported that methanolic and ethanolic extracts of *S. dolichosepalum* showed a slight inhibitory effect against *S. aureus, Salmonella spp., A. hydrophila*, and *P. aeruginosa*. However, the effect was not enough to be considered significant and the microorganisms showed resistance to these extracts. Out of the two types of extracts used, ethanolic was the most active on *S. aureus, Salmonella spp.,* and *A. hydrophila,* while the methanolic extract was most effective against *P. aeruginosa.* The results of this research, and those already mentioned, confirm the antimicrobial activity of the leaves of *S. dolichosepalum*.

MIC values for *S. dolichosepalum* extracts tested on *C. albicans* and *F. oxysporum* are shown in Figure 1. It was shown that the acetone extract was the most effective against the two microorganisms, with values ranging from 11.3 to 34.2 mg/mL. *C. albicans* was more sensitive to chloroform, ethanolic, and acetone extracts, while *F. oxysporum* was more sensitive to acetone, dichloromethane, and methanolic extracts. The ANOVA analysis performed was significant for both microorganisms, resulting in probability values of 0.0005 and 0.0010 for *C. albicans* and *F. oxysporum* respectively, indicating different effects for each type of extract on the two microorganisms. On the contrary, in the publication derived from the preliminary results, values of 387.9 and 311.6 mg/mL were found for *S. dolichosepalum* acetonic and chloroform extracts, respectively (Martin *et al*., 2016). There are two research reports that mention the antifungal effect of extracts of *S. dolichosepalum* against *C. albicans*. Arango *et al.* (2004) determined that the ethanolic extract had a MIC value of 250 mg/mL, a higher value than those found in this research; a possible explanation lies in the climatic and environmental

differences where the plant grew, affecting biosynthesis and the accumulation of secondary metabolites. In 2006, it was found that aqueous extracts of *S. dolichosepalum* did not inhibit this microorganism (Marin *et al*., 2006).

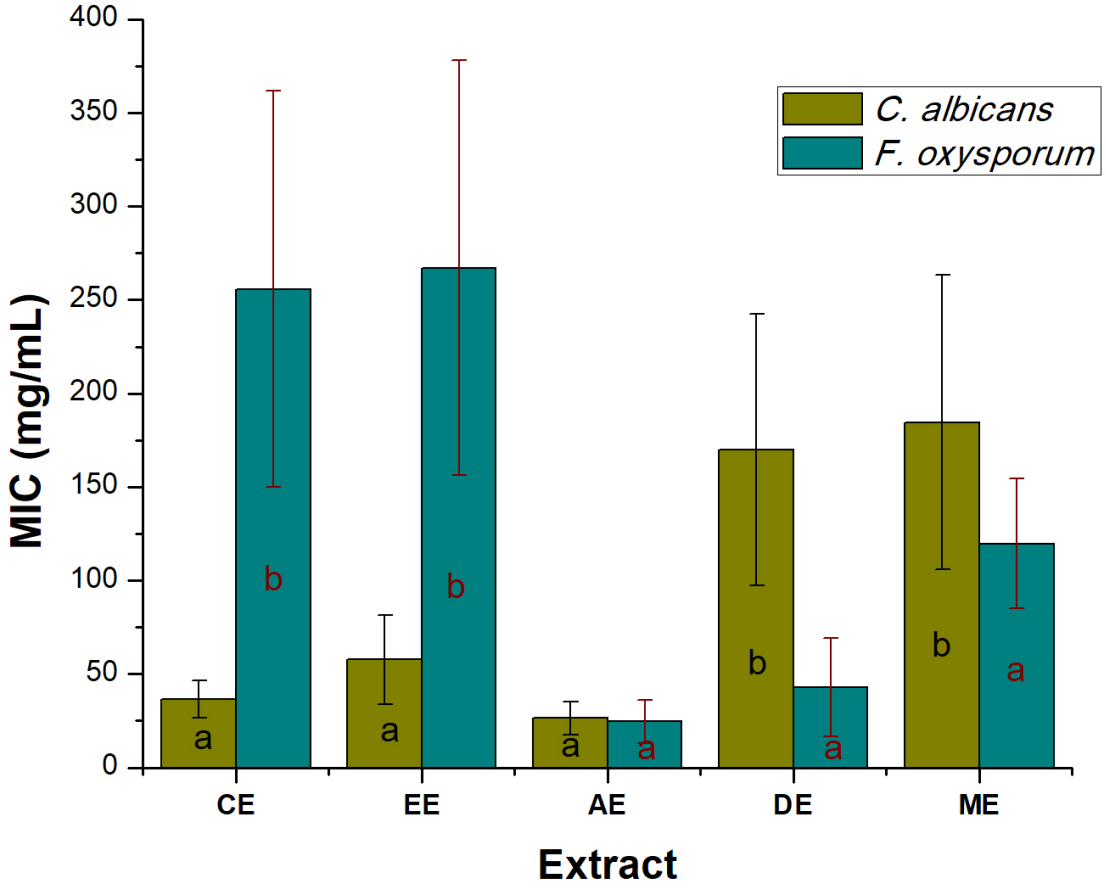

FIGURE 1 - Minimum Inhibitory Concentration for each extract and fungus. The data are shown as mean ± SD (n = 4). Different letters indicate statistical significance at 5% according to the Duncan test.

Antifungal activity: Fractions

All the analyzed fractions showed antifungal activity against the inoculated microorganisms. The results obtained for the fractions are found in Table II, where it is observed that the acetone fraction was the most effective, followed by dichloromethane and hexane. The data are statistically significant ($p < 0.0001$) for *F. oxysporum* and *C. albicans*, the first being more sensitive to the inhibitory effect of the compounds present in each fraction, similar to the crude extracts. The acetone fraction was determined to be the most effective, with MIC values of between 3.5 to 5.8, and 5.8 to 9.7 mg/mL for *F. oxysporum* and *C. albicans* respectively. It was also found that *F. oxysporum* was the microorganism that was the most sensitive to the effect of the compounds present in the fractions (figure 2).

TABLE II - Zones and inhibition percentages produced by each fraction (950 mg/mL). The data are shown as mean ± SD (n = 4). Different letters in the same column differ statistically at 5% according to the Duncan test

	F. oxysporum		*C. albicans*	
	IZ (mm)	**% I**	**IZ (mm)**	**% I**
AF	16 ± 2[b]	105.5 ± 15.5[b]	15 ± 2[b]	105.8 ± 15.4 [b]
DF	6 ± 2[a]	32.8 ± 12.0[a]	3 ± 1[a]	19.1 ± 6.6 [a]
HF	3 ± 1[a]	19.1 ± 6.6[a]	3 ± 1[a]	17.3 ± 6.9 [a]
FLU	13 ± 1		14 ± 2	

AF: acetone fraction; **DF:** dichloromethane fraction; **HF:** hexane fraction. **FLU:** fluconazole; **%I:** inhibition percentage; IZ: inhibition zone

Few investigations have used fractions of extracts of plants from the genus Solanum to inhibit the growth of these microorganisms. Das, Lahan, Srivastava, (2010) reported the use of fractions of petroleum ether, chloroform, methanol, and water as antifungals against *C. albicans*. These authors found inhibition halos and percentages of inhibition that suggest that these *S. melongena* fractions have antifungal potential, with the exception of the aqueous fraction.

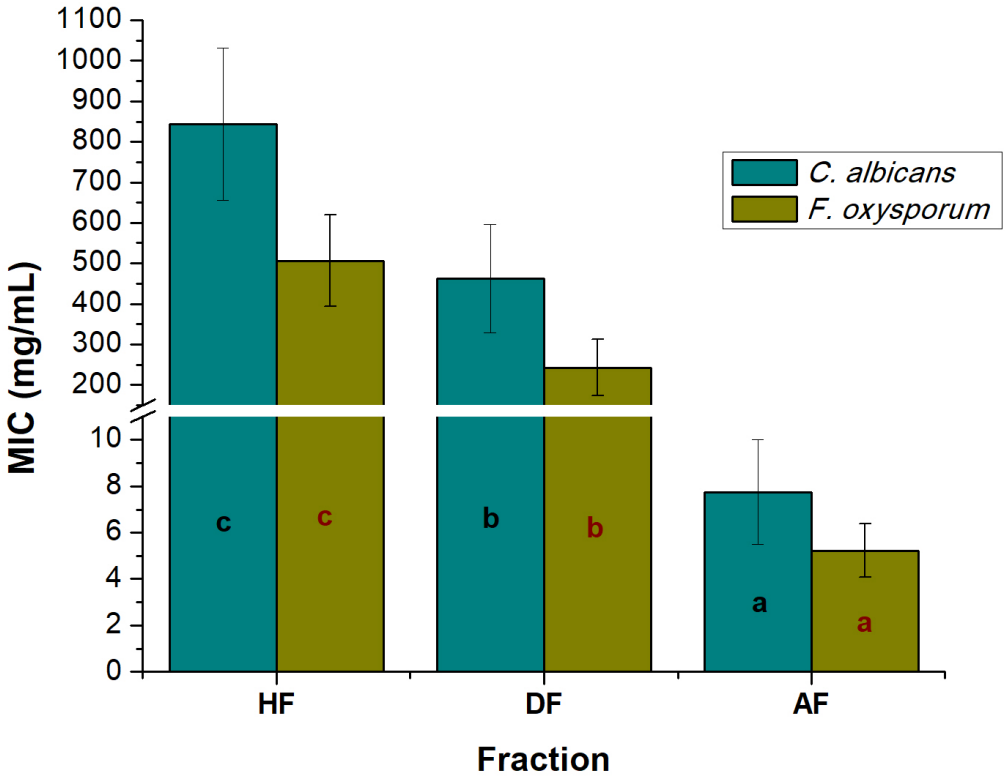

FIGURE 2 - Minimum Inhibitory Concentration for each fraction and fungus. The data are shown as mean ± SD (n = 4). Different letters indicate statistical significance at 5% according to the Duncan test.

Antioxidant Activity: Crude extracts

The results found the phenolic compounds present in the *S. dolichosepalum* plant show a greater affinity for acetone, followed by methanol, ethanol, chloroform, and dichloromethane (Table III). It is statistically significant ($p<0.001$), and the only similar effect is for chloroform and dichloromethane extracts. Similar results were found by Alothman, Bhat, Karim (2009). They analyzed the effects of different solvents and mixtures for the extraction of phenolic compounds in guava (*Psidium guajava*), pineapple (*Ananas comosus*), and banana (*Musa paradiasaca*) fruits. They report higher contents when acetone and its aqueous mixtures are used, compared to ethanol, methanol, water, and their respective mixtures. They explain the average polarity of acetone (dielectric constant: 21 for acetone, 33 for methanol, 25 for ethanol, 9 for dichloromethane, and 5 for chloroform), which facilitates the extraction of phenolic compounds. Guzmán *et al*. (2016) determined that the aqueous and ethanolic extracts of *S. marginatum* leaves have lower values than those of *S. dolichosepalum* (0.058 and 0.052 mg GAE/g d.s.. By contrast, Zadra *et al*. (2012) determined a phenolic compound content of 259.95 mg GAE/g in aqueous extracts of leaves of *S. guaraniticum*, 2 to 8 times higher than those found for *S. dolichosepalum*.

TABLE III - Antioxidant activity of *S. dolichosepalum* extracts. Mean ± SD (n = 3). Duncan test, different letters in the same column differ statistically at 5%

	TAA (mmol TE/L)		Percentages of inhibition		IC_{50} (mg/mL)		TPC (mg GAE/g DS)
	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	
AE	0.241±0.030^{d}	0.260±0.011^{d}	53.9±3.7^{d}	83.6±3.5^{d}	2.3	1.4	98.4±2.7^{d}
ME	0.199±0.012^{c}	0.207±0.024^{c}	50.1±3,3^{c}	63.8±7.4^{c}	3.8	1.9	69.7±2.2^{c}
EE	0.100±0.010^{b}	0.171±0.022^{b}	25.5±2.3^{b}	56.5±6.8^{b}	4.1	3.1	37.2±1.1^{b}
CE	0.043±0.023^{a}	0.050±0.016^{a}	10.1±4.2^{a}	19.7±4.7^{a}	57.4	17.9	8.8±3.6^{a}
DE	0.038±0.015^{a}	0.041±0.003^{a}	7.0±0.7^{a}	16.9±0.7^{a}	*	1.4	4.7±1.9^{a}

AE: Acetone extract; ME: methanolic extract; EE: ethanolic extract, CE: chloroform extract; DE: dichloromethane extract; IC_{50}: 50% inhibitory concentration; TPC: total phenolic compounds; TAA: total antioxidant activity. * not found by opalescence.

The percentages and Trolox equivalent values for ABTS and DPPH reported are statistically significant ($p<0.001$). It was found that the acetone and methanolic extracts generated greater radical inhibition compared to ethanol, chloroform, and dichloromethane. These results infer a relationship between antioxidant activity and the content of phenolic compounds, as also mentioned in the literature (Choi *et al*., 2011; Quideau *et al*., 2011; Zadra *et al*., 2012). Priyadharshini and Sujatha (2013) reported higher inhibition percentages for acetone extracts, compared to those of ethyl acetate, methanol, and hexane, given the higher contents of total phenolic compounds found in extracts of acetone and ethyl acetate from the *S. erianthum* plant.

ANOVA analysis of IC_{50} generated statistical differences ($p<0.001$) for the radicals, with the acetone extract being the lowest value, confirming that it is the extract with the highest antioxidant power, followed by the methanolic, ethanolic, chloroform, and dichloromethane extracts, respectively. It was not possible to determine the IC_{50} value with DPPH for the dichloromethane extract, since, by increasing the concentration of the extract, a proportional increase in the percentage of inhibition was not verified, on the contrary, it decreased due to the effects of red opalescence generated by the amount of extract present and/or by the absorption generated of compounds with λmax similar to DPPH. Similar results were found by Magalhães *et al.* (2014), who report that the ethanolic

and aqueous extracts of *S. thomassifolum* showed lower IC_{50} values compared to hexane and ethyl acetate extracts for ABTS and DPPH. Priyadharshini and Sujatha (2013) also found that the lowest IC_{50} values occurred with the acetone extract (0.156 mg/mL), followed by ethyl acetate and methanol (1,122 and 1,746 mg/mL) when analyzing extracts obtained from *S. erianthum* as inhibitors of the stable radical DPPH.

Antioxidant activity: Fractions

TPC was statistically significant ($p<0.001$), with the acetone fraction containing the highest content (Table IV). If these values are compared with those obtained for the crude acetone extract, the primary separation, eluting with hexane and dichloromethane, eliminated secondary metabolites of low polarity, possibly carotenoids (hexane fraction), and others of a slightly higher polarity, from the soluble fraction of acetone. The content in the acetone fraction was on average 109.0013 mg GAE/g d.s., while the crude extract was 98.4376 mg GAE/g d.s..

TABLE IV - Antioxidant activity of the fractions of the acetone extract of *S. dolichosepalum*. Mean ± SD (n = 3). Duncan test, different letters in the same column differ statistically at 5%

	TAA (mmol TE/L)		Percentages of inhibition		IC_{50} (mg/mL)		TPC (mg GAE/g DS)
	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	
AF	0.321 ± 0.000	0.294 ± 0.008	80,6 ± 0.3	93,9 ± 2.5	2.1	0.8	109.0 ± 5.4
DF	0.015 ± 0.000	0.026 ± 0.004	4,1 ± 0.5	12,3 ± 1.3	*	24.1	5.1 ± 0.2
HF	0.013 ± 0.002	0.010 ± 0.006	3.6 ± 0.6	7.5 ± 1.8	*	*	2.1 ± 0.1

AF: acetonic fraction; DF: dichloromethane fraction; HF: hexane fraction; *: could not be determined by the opalescence produced by the concentration of the fraction.

Few data are reported for total phenolic compounds in fractions of isolated extracts of species of the genus Solanum. However, Zadra *et al.* (2012) determined the content of total phenolic compounds in different fractions obtained from chloroform extracts of *S. guariniticum*. They found values of 195.90 ± 1.24, 546.57 ± 2.35, and 259.82 ± 2.17 mg GAE/g for fractions of ethyl acetate, butanol, and chloroform respectively, values higher than for the crude chloroform extract (259.95 ± 0.69 mg GAE/g) and the fractions of the acetone extract of *S. dolichosepalum*. In this research, it is noted that the affinity of the phenolic compounds is different from the metabolites present in the acetone extract of *S. dolichosepalum*

Inhibition percentages ranged from 2.93 to 80.83 (DPPH) and 6.34 to 96.65% (ABTS). ANOVA was significant ($p < 0.001$) for both variables and it was the acetone fraction which showed the highest values. The values obtained in the hexane and dichloromethane fractions of the acetone extract of *S. dolichosepalum* are similar, as opposed to the acetone fraction that produced greater inhibitions (80.73%). In 2012, Zadra *et al.* (2012) used chloroform, butanolic, and ethyl acetate fractions of *S. guariniticum*, finding inhibition percentages of 90%, 82%, and 68%, respectively.

HPLC-DAD qualitative analysis

In this analysis, 10 of the 24 tested compounds were identified. The presence was found of *p*-hydroxybenzoic, vanillic, ferulic, *trans*-cinnamic, caffeic, *p*-coumaric, and rosmarinic acids, the xanthine theobromine, and the flavonoids quercetin and luteolin (Table V). It was observed that the extracts or fractions of greater polarity presented a greater number of compounds, many of which could not be identified. The hexane fraction was the only sample that showed no presence of any of the 24 analyzed substances.

The presence in other plant species of the genus Solanum of the same compounds identified in *S. dolichosepalum* is mentioned in the literature. Below are the investigations where some of these substances have been reported. In the fruit of the tree tomato (*Solanum betaceum*) *p*-Hidroxybenzoic is found (Potawale *et al*., 2008), as well as in the *S. nigrum* plant (Vasco *et al*., 2009). For its part, ferulic acid is present in the leaves of *S. nigrum* (Huang, Syu, Lin, 2010), potato peel (*S. tuberosum*) (Ieri *et al*., 2011), and the fruit of *S. betaceum* (Vasco *et al*., 2009). Caffeic acid is a phenolic compound present in different species of the genus Solanum, including the leaf of *S. nigrum* (Huang *et al*., 2010), the fruit of *S. betaceum* (Vasco *et al*., 2009), and *S. tuberosum* (Andre *et al*., 2007). In *S. nigrum* (Huang *et a*l., 2010) and *S. betaceum* (Vasco *et al*., 2009), *p*-Coumaric acid has been found. In addition to phenolic acids, extracts and fractions of *S. dolichosepalum* contain quercetin, a flavonoid that also has an important distribution in Solanum species. Some investigations report its presence in plants such as *S. nigrum* (Wang *et al*., 2010), S. *betaceum* (Vasco *et al*., 2009), and *S. indicum* (N'Dri *et al*., 2010).

TABLE V - Compounds identified in extracts and fractions of *S. dolichosepalum*

Compound	t_R (min)	AE	ME	EE	CE	DE	AF	DF	HF
Theobromine	3.58	+	-	-	-	-	-	+	-
***p*-Hidroxybenzoic acid**	6.85	+	+	+	+	-	+	-	-
Vanillic acid	10.57	-	-	-	+	+	-	-	-
Caffeic acid	11.92	+	+	+	+	+	+	-	-
***p*-coumaric acid**	17.16	+	+	+	-	-	+	-	-
Ferulic acid	18.68	+	-	+	+	+	+	+	-
Rosmarinic acid	22.01	+	-	+	-	-	+	-	-
***trans*-cinnamic acid**	25.37	+	-	-	-	+	-	+	-
Quercetin	26.86	+	+	+	-	-	+	-	-
Luteolin	27.17	-	+	-	-	-	-	-	-

t_R: retention time; AF: acetone fraction; DF: dichloromethane fraction; HF: hexane fraction; AE: Acetone extract; ME: methanolic extract; EE: ethanolic extract, CE: chloroform extract; DE: dichloromethane extract. +: present; -: absence

Phenolic compounds that show antifungal activity against different pathogens are mentioned in different studies. An example is raised by Alves *et al.* (2014) who tested the effects of four phenolic compounds (quercetin, gallic acid, luteolin, and catechin) against different *Candida* species (*albicans, glabrata, parapsilosis*, and *tropicalis*) finding that they all have important effects on the species analyzed. Other research reports that flavanols and falvan-3-oles are effective in inhibiting the growth of *C. albicans* (Daglia, 2012), as is chlorogenic acid (Lee

et al., 2008). According to the data found in this research and the reports of other studies, it can be inferred that the antifungal activity of extracts and fractions of *S. dolichosepalum* can be explained, in part, by the presence of phenolic compounds.

Correlations

In the literature, the relationship between the content of total phenolic compounds and the antioxidant capacity of extracts is mentioned (Brown, 2005; Choi *et al*., 2011; Quideau *et al*., 2011; Zadra *et al*., 2012), but it is much lower between the inhibition of microorganisms and the content of phenolic compounds or the antioxidant activity. Table VI shows in detail the Pearson correlation coefficients (PCC) found for each variable of interest. It was observed that the correlation was positive between the content of total phenolic compounds (TPC) and the inhibition of the DPPH radical (DPPH), the inhibition of ABTS and TPC, and the inhibition of ABTS with DPPH. These data show the direct proportionality between these variables.

TABLE VI - Pearson correlation coefficients for the involved variables

	TPC	DPPH	ABTS	IC_{50}ABTS	IC_{50}DPPH	IHFO	IHCA	MICCA	MICFO
TPC	1	0.988**	0.976**	-0.861**	-0.482*	0.844**	0.808**	-0.549**	-0.654**
DPPH	0.988**	1	0.967**	-0.834**	-0.459	0.810**	0.840**	-0.564**	-0.647**
ABTS	0.976**	0.967**	1	-0.915**	-0.525*	0.881**	0.776**	-0.634**	-0.673**
IC_{50}ABTS	-0.861**	-0.834**	-0.915**	1	0.363	-0.782**	-0.602**	0.589**	0,418
IC_{50}DPPH	-0.482*	-0.459	-0.525*	0.363	1	-0.519*	-0,205	-0.232	0.648**
IHFO	0.844**	0.810**	0.881**	-0.782**	-0.519*	1	0.742**	-0.609**	-0.628**
IHCA	0.808**	0.840**	0.776**	-0.602**	-0.205	0.742**	1	-0.484*	-0.579**
MICCA	-0.549**	-0.564**	-0.634**	0.589**	-0.232	-0.609**	-0.484*	1	0.773**
MICFO	-0.654**	-0.647**	-0.673**	0.418	0.648**	-0.628**	-0.579**	0.773**	1

TPC: Total phenolic compounds content. **DPPH and ABTS**: total antioxidant activity of DPPH and ABTS radicals. **IC_{50}ABTS and IC_{50}DPPH:** 50% inhibitory concentration of ABTS and DPPH. **IHFO and IHCA:** percentages of inhibition against *F. oxysporum* and *C. albicans*. **MICCA and MICFO:** minimum inhibition concentration for *C. albicans* y *F. oxysporum*. ** Correlation is significant at the 0.01 level (bilateral). * The correlation is significant at 0.05 (bilateral).

In many studies, it is mentioned that the antioxidant activity of natural product extracts is mainly due to the presence of phenolic compounds (Baño *et al*., 2003; Choi *et al*., 2011; Floegel *et al*., 2011; Quideau *et al*., 2011), and to a lesser degree, of lipophilic antioxidants, such as carotenoids and fat-soluble vitamins (Dragovic *et al*., 2007; Thaipong *et al*., 2006). Thaipong *et al.* (2006) also found, as did the study with *S. dolichosepalum*, that the ABTS radical had a higher correlation with the content of phenolic compounds (PCC = 0.970) for methanolic extracts *of P. guajava*. L. Floegel *et al.* (2011) found similar results between the phenolic compounds, ABTS and DPPH. For those authors, ABTS presents a greater correlation with the content of phenolic compounds compared with DPPH, when studying these variables in different antioxidant fruits of the United Kingdom. In addition, they also reported a positive correlation between DPPH and ABTS. On the contrary, Sulaiman *et al.* (2011) found no significant correlation between the content of phenolic compounds and the antioxidant activity of DPPH and FRAP but coincided in finding positive correlations between DPPH and FRAP. In this investigation, a positive

correlation was found for the antioxidant effect of *S. dolichosepalum* extracts for ABTS and DPPH.

Another of the variables analyzed was the concentration needed to inhibit 50% of the radical. Values of PCC show a negative correlation (inverse proportionality) between the content of total phenolic compounds with IC_{50} ABTS (-0.836, $p < 0.001$) and IC_{50} DPPH (-0.482, $p = 0.043$), the correspondence being lower with the values for DPPH. It should be taken into account that this variable could not be calculated for the dichloromethane extract, hexane fractions, and dichloromethane, affecting the Pearson coefficient calculations. On the other hand, it was found that the inhibition halos produced by the extracts and fractions on the microorganisms studied have a positive correlation with the content of total phenolic compounds (Table VI). The best relationship was found for *F. oxysporum*, which turned out to be the microorganism that was the most sensitive to the effect of extracts and fractions of *S. dolichosepalum*. The percentages of inhibition also showed positive correlations (directly proportional) with the content of total phenolic compounds with PCC of 0.844 and 0.808 ($p<0.001$) for *F. oxysporum* and *C. albicans,* respectively. With these data, it can be inferred that the antifungal activity against the two fungi can be due to the number of phenolic compounds present in the extracts and fractions.

CONCLUSION

All the extracts and fractions presented antioxidant activity, the acetone extract and its acetonic fraction being those that generated greater stabilization of the DPPH and ABTS radicals. Also, the content of total phenolic compounds suggests a greater affinity of acetone with the phenolic compounds present in the plant *S. dolichosepalum*. The extracts used showed antifungal activity against *C. albicans* and *F. oxysporum*. *F. oxysporum* was the microorganism that was most sensitive to acetone and ethanolic extracts, whereas *C. albicans* was more sensitive to methanolic and acetonic extracts. The fractions obtained inhibited the growth of the microorganisms used, finding statistical differences between them, with the acetone fraction being the most active. The presence of *p*-hydroxybenzoic, vanillic, ferulic, *trans*-cinnamic, caffeic, *p*-coumaric, and rosmarinic acids, theobromine, quercetin, and luteolin was found. It was determined that the content of total phenolic compounds is directly proportional to the inhibition of the radicals ABTS and DPPH, and the inhibition of the microorganisms analyzed. The best adjustments were presented between TPC with ABTS and DPPH. It was also found that the IC_{50} of the two radicals and the MIC of the two fungi present inverse proportionality. These correlations showed that the content of total phenolic compounds affects all the variables analyzed. According to the data found in this research and the reports of other studies, it can be inferred that the antifungal and antioxidant activities of extracts and fractions of *S. dolichosepalum* can be explained, in part, by the presence of phenolic compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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